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Mechanistic *in vitro* studies: What they have told us about carcinogenic properties of elongated mineral particles (EMPs)

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ABSTRACT

In vitro studies using target and effecter cells of mineral-induced cancers have been critical in determining the mechanisms of pathogenesis as well as the properties of elongated mineral particles (EMPs) important in eliciting these responses. Historically, *in vitro* models of 'mutagenesis' and immortalized cell lines were first used to test the theory that EMPs were mutagenic to cells, and 'genotoxicity', as defined as damage to DNA often culminating in cell death, was observed in a dose-dependent fashion as responses of many cell types to a number of EMPs. As two-stage and multi-step models of cancer development emerged in the 1970s and 1980s, differentiated 3D organ cultures and monolayers of lung epithelial and mesothelial cells were used to probe the mechanisms of cancer development. These studies demonstrated a spectrum of pre-neoplastic changes, including hyperplasia and squamous metaplasia, in response to long (> 5 μm in length) needlelike EMPs whereas long, curly chrysotile fibers caused acute cytotoxicity. Shorter fibers of many types were taken up by cells and encompassed in phagolysosomes. Comparative studies using chemical carcinogens showed that chemical agents interacted directly with DNA whereas long EMPs appeared to be promoters of cancers *via* a number of mechanisms such as inflammation, generation of oxidants, and instigation of cell division. The multitude of these signaling cascades and epigenetic mechanisms of both lung cancers and mesotheliomas have been most recently studied in normal or telomerase immortalized human cells. Importantly, many of these pathways are elicited by long, straight amphibole asbestos fibers or carbon nanotubes in rodents and not by short (< 5 μm) EMPs, fragments, or nonfibrous particles. However, the chemistry and surface properties of long fibers are also critical in cell responses to minerals.

1. Introduction

Cell and organ cultures are valuable in elucidating mechanisms of cancer causation by EMPs and the properties of EMPs that elicit these effects. Unlike epidemiologic studies where workers are exposed to a variety of EMPs, often in a number of workplace settings over time, known quantities of EMPs with defined properties can be introduced to cell cultures in a set regimen, allowing dose-response experiments. Moreover, various EMPs can be assessed comparatively over time. Although most normal cell culture models are short-term in nature, *i.e.*, hours or days, organ cultures and immortalized cells have allowed exposures for as long as months. Moreover, cells or tissues can also be implanted or injected into syngeneic or immune-deficient animals over their lifetime to assay their tumorigenic potential.

The purpose of this review is to highlight what has been learned over time about mechanisms of cancer causation. How these general mechanisms have been applied to the development of *in vitro* models to assess critical stages in the development of lung cancers and mesotheliomas by EMPs is then described. How this information has led to an understanding of the properties of EMPs critical to mesothelioma development is emphasized. Since many laboratories are examining the possibility of using *in vitro* assays in place of or in combination with *in vivo* studies, a short discussion is also included on the limitations and differences between these models in assessing health effects of EMPs.

2. General concepts of cancer development

The development and use of *in vitro* models over time has corresponded with the evolution of research and knowledge on cancer etiology in humans (Degregori, 2017; Tomasetti et al., 2017; Tomatis and Huff, 2002). While some scientists have suggested that the relative contributions of DNA replications and mutations are overwhelming drivers of cancer risk, others argue that experimental and evolutionary data point to tissue microenvironment and epigenetic changes as being key to tumorigenesis.

The age of chemical carcinogenesis began in the 1940s and has

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persisted for decades. This time span reflects a lengthy progression from initially published scientific observations that chemical carcinogens in the environment were carcinogenic in animals to the official recognition that they caused cancers in humans. A two-stage hypothesis that proposed sequential initiating and promoting stages of cancer in mouse papillomas after painting of mouse skin with cigarette tars or the polycyclic aromatic hydrocarbon, benzo(a)pyrene, was endorsed by Berenblum (Berenblum, 1974) and reproduced consequently in the lung and other organs. In the two-step model, 'mutations' were observed as one of the first signatures of initiating agents, whereas 'tumor promotion' induced by irritants and other compounds was characterized as a series of epigenetic events manifested as proliferation and inflammation of mutated cells. The fact that many chemicals are mutagens that act directly on DNA or are metabolized to forms that can interact with or form adducts with DNA, gave rise to the hypothesis that most carcinogens were mutagens that could be evaluated for their potency in the Salmonella/microsome test model (Ames et al., 1973). The conviction that most chemical carcinogens were mutagens due to an alteration of DNA was largely supported by testing of soluble chemicals in this assay. Thus, the first stage or 'initiation' of cancers was deemed an irreversible effect attributed to heritable mutations in DNA where the second stage, 'promotion', appeared to be nongenetic or epigenetic, defined decades ago as processes not involving interactions with DNA. The complexity of tumor promoting events and the fact that multiple genetic and nongenetic events often occurred during the long latency period of most tumor types gave rise to the contemporary multistep model of tumor progression. Carcinogenesis is regarded today as a stepwise series of events favoring increased genomic instability of cells during which they acquire invasive and metastatic properties. During tumor progression, premalignant cells are rapidly dividing, and errors in replication and DNA repair occur. A number of proto(o)oncogenes and tumor suppressor genes have been identified that mediate multiple pathways involved in both genetic and epigenetic events during tumorigenesis. Often these genes and their encoded proteins modulate a number of extracellular and intracellular receptors in premalignant cells to stimulate a number of pathways necessary for malignant tumor development. It is known now that multiple mutations can occur during tumor development that contribute to hundreds of properties required for cell transformation. For example, in epithelial cell tumors, both genetic and epigenetic events govern sequential phenotypic changes from hyperplasia to metaplasia, to dysplasia, and finally, malignancy (carcinoma).

The modern-day definition of 'epigenetic' mechanisms has evolved over time to encompass the fact that alterations in the primary structure of DNA do not underlie most changes in the development of tumors. Accordingly, "an epigenetic trait can be a stable inheritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" (Berger et al., 2009). Multiple, sometimes reversible, epigenetic mechanisms are recognized including DNA methylation, histone modifications, and effects by noncoding RNAs, a class of regulatory molecules that control gene expression by binding to complementary sites on target messenger RNA (mRNA) transcripts. Noncoding RNAs can be long (lncRNAs) or short (miRNAs) (reviewed in Robb et al., 2017). The latter can bind to complementary protein-coding RNA sequences to induce an RNA-mediated (RNAi) interference pathway whereby mRNA targets are cleaved and silenced. Alternatively, miRNAs can bind to imperfect complementary sites within the 3′-untranslated regions (3'UTR) of target protein-coding mRNAs to repress gene expression at the level of translational control. One miRNA can thus influence expression of multiple mRNAs. Studies from a number of laboratories have shown that downregulation of certain miRNAs are observed in a number of human cancers, suggesting that they may function as tumor suppressor genes. Others function in existing oncogene pathways and can control cell differentiation and programmed cell death or apoptosis. Thus, understanding the epigenetic mechanisms important in tumor development are vital in current strategies to inhibit tumor progression and growth. Their exploration in asbestos-induced mesotheliomas is burgeoning with the goals of establishing biomarkers and treatment strategies in mesotheliomas (Mossman, 2017; Reid, 2015).

It was discovered decades ago that foreign bodies such as plastic implanted under the skin of animals gave rise to sarcomas (Brand et al., 1976). Other cancers were linked to repeated infections and scarring of tissues. The theory that mesotheliomas and lung cancers might be categorized as tumors linked to foreign body perturbations by durable needle-like fibers is an attractive one. That long (> 5 μm) amphibole asbestos fibers induce chronic inflammation in lung and pleura because they persist at sites of mesothelioma development has been shown by several laboratories (Boutin et al., 1996; Goodglick and Kane, 1990; Moalli et al., 1987; Murphy et al., 2013; Murphy et al., 2012). This view has been spear-headed by observations that long thin fibers, *i.e.*, carbon nanotubes and amosite asbestos, are trapped at stomata at the pleural or peritoneal surface (Donaldson et al., 2010; Murphy et al., 2011; Schinwald et al., 2012). Human mesothelial cells can initiate an autocrine pathway of inflammation *via* inflammasome activation in response to long amphibole fibers and erionite (Hillegass et al., 2013; Sayan and Mossman, 2016).

3. Asbestos interactions with DNA and chromosomes

In line with the early observations that chemical carcinogens interacted with DNA to cause mutations, asbestos fibers, classified as human carcinogens by IARC and other agencies regardless of fiber type, were tested in rodent cell culture models of mutagenesis and transformation. In the 1970s and 1980s, many investigators failed to identify the type and source of asbestos they were using and frequently used only one concentration of fibers, rendering interpretation of data difficult. Although crocidolite and chrysotile asbestos fibers and synthetic vitreous fibers tested positively in the Syrian hamster cell transformation assay, asbestos fibers of a variety of types tested negatively in other models of mutagenesis and transformation (Daniel, 1983; Denizeau et al., 1985; Dipaolo et al., 1983; Jaurand et al., 1986; Oshimura et al., 1984; Palekar et al., 1988; Reiss et al., 1982; Shelby, 1988; Sincock et al., 1982). The lack of asbestos effects in these models was often attributed to the fact that asbestos fibers could not penetrate the cell wall of bacterial cells and were found in the cytoplasm as opposed to the nucleus of mammalian cells (Mossman et al., 1977). For these reasons, asbestos was categorized as an agent that did not directly interact with DNA (Shelby, 1988; Williams, 1979). In a unique hamster-human cell model, gene mutations by chrysotile asbestos occurred at lethal concentrations of fibers and were characterized by large deletions in DNA incompatible with cell viability and proliferation (Hei et al., 1992). These results and subsequent research (reviewed in Shukla et al., 2003) showed that mutational events at high, 'cell-killing' concentrations of asbestos fibers were due to the production of reactive oxygen species (ROS) when normal antioxidant defense mechanisms were overwhelmed. Recent work has indicated that amosite asbestos-induced ROS production in human alveolar epithelial cells is from mitochondrial, rather than from nuclear sources (Kim et al., 2014). Thus, asbestos fibers do not interact with DNA directly to cause heritable mutations or cell transformation but may generate ROS *via* other cellular pathways that are linked to cell death.

'Cytotoxicity' or cell death is a signature of asbestos effects in many studies although the relationship of cytotoxicity to carcinogenicity is speculative (reviewed in Mossman and Begin, 1989). Another difficulty in interpretation of *in vitro* studies is that chrysotile asbestos is more toxic than various types of amphibole asbestos or glass fibers when fibers are compared on an equal weight basis. Often, trends in toxicity by different fiber types are different if toxicity is measured as fiber numbers per cell. The cytotoxic effects of chrysotile have been attributed to its positive surface charge (Mossman et al., 1983a) rendered by Mg++. Surface charge was modified by the acidic environment of

lysosomes in epithelial and mesothelial cells that also caused leaching of Mg++ (Craighead et al., 1980; Jaurand et al., 1977; Jaurand et al., 1984). Regardless of fiber type, long fibers (> 5 μm) were more toxic to cells than equal mass equivalents of short fibers, observations correlating with the inflammatory potential of long fibers after injection into rodents (Donaldson et al., 1989; Goodglick and Kane, 1990; Hart et al., 1994; Wright et al., 1986) and a compendium of data showing that, regardless of route of administration, long (> 5 μm) fibers were more carcinogenic and fibrogenic than shorter fibers (Berman et al., 1995; Donaldson et al., 2010; Spurny et al., 1979; Stanton et al., 1981).

Studies in the 1980s and early 1990s reported aneuploidy and chromosomal damage by asbestos and other fiber types. Investigators explored the importance of fiber type, fiber length, and dose. In studies exploring dose-related changes in cell responses, thresholds were indicated below which aberrations were not seen (Dipaolo et al., 1983; Jaurand et al., 1986; Mikalsen et al., 1988; Oshimura et al., 1984; Palekar et al., 1988; Price-Jones et al., 1980). These results suggested cell repair mechanisms that were later elucidated as DNA repair enzymes and induction of antioxidants. The importance of fiber length was attributed to physical interactions of long fibers (> 15 μm) with the mitotic spindle of aberrantly dividing cells and inhibition of cytokinesis (Jensen and Watson, 1999). However, glass fibers also interacted with chromosomes and caused aneuploidy and morphological transformation of rodent cells, questioning the relevance of these findings to carcinogenesis in man. The classical rodent studies by Stanton et al. (Stanton et al., 1981) and Pott (Pott, 1978) also support the conclusion that long (> 8 μm), thin (< 0.25 μm wide) fibers of a number of types cause pleural sarcomas and mesotheliomas regardless of chemical composition and durability. Although proliferation of mesothelial cells has been demonstrated in rodent inhalation studies in response to crocidolite and chrysotile asbestos (Mossman et al., 2011; Quinlan et al., 1995; Shukla et al., 2004), interaction of asbestos fibers with mitotic cells or chromosomes has not been observed *in vivo*.

Experiments using tracheobronchial epithelial cells as models of lung cancer development showed that human lung epithelial cells were resistant to DNA damage by asbestos (Kodama et al., 1993; Lechner et al., 1985). Other studies demonstrated that crocidolite and chrysotile asbestos fibers did not interact directly with or break DNA (Eastman et al., 1983; Mossman et al., 1983b).

4. Cell proliferation and activation of signaling cascades

In the 1980s, it was demonstrated that crocidolite and chrysotile asbestos fibers acted mechanistically on tracheal epithelial and mesothelial cells, as did the classical tumor promoters, phorbol esters (reviewed in Mossman et al., 1985). In tracheal organ cultures, long fibers (> 5 μm) were associated with the development of hyperplasia and squamous metaplasia whereas fragments of minerals were inactive (Mossman et al., 1980; Woodworth et al., 1983). In several of these studies, nonfibrous and short (primarily < 5 μm) fragments of riebeckite or antigorite, were used to demonstrate the importance of long needlelike shape in parameters of tumor development. It was frequently observed that cells proliferated over the surfaces of long nontoxic amphibole or synthetic vitreous fibers whereas long chrysotile fibers caused cell death (Craighead et al., 1980; Woodworth et al., 1983).

Since the theory that increased cell proliferation was a cause of human cancers was gaining popularity (Preston-Martin et al., 1990), the mechanisms of cell division of asbestos fibers were studied using a number of bioassays (Heintz et al., 1993; Landesman and Mossman, 1982; Mossman et al., 2011; Ramos-Nino et al., 2003). Like in assays exploring aneuploidy by asbestos and other long fibers, long fibers of a number of types including synthetic vitreous fibers induced parameters of cell proliferation. These included increased cell division, synthesis of polyamines or growth regulatory proteins that are increased in cells before cell division occurs, and increased gene and protein expression of *fos* and *jun* protooncogenes (Heintz et al., 1993; Janssen et al., 1994a; Landesman and Mossman, 1982; Marsh and Mossman, 1988). Studies using fibers over a range of concentrations demonstrated thresholds below which no increases in gene expression and/or cell division occurred (Heintz et al., 1993; Lemaire et al., 1986; Sesko and Mossman, 1989).

With the advent of redox chemistry, collaborations with chemists and geologists spurred collaborative research efforts to gain an understanding of how mesotheliomagenic amphibole fibers catalyzed oxidative damage to cells (Guthrie Jr. and Mossman, 1993). These investigations were important in elucidating that: 1) iron content and its surface availability were increased in crocidolite and amosite asbestos; 2) crocidolite and amosite fibers caused intracellular mobilization of iron and activation of iron transport receptors in cells; 3) DNA damage and lipid peroxidation were induced by oxidants *via* iron-dependent reactions; and 4) activation of alveolar and peritoneal macrophages increased production of extracellular oxidants after exposures to asbestos and other long (> 5 μm) fibers (reviewed in Shukla et al., 2003). In response to fibers, both lung epithelial and mesothelial cells showed elevations and activation of a number of antioxidant pathways that could prevent cell injury and other signatures of oxidant stress if added exogenously to cell cultures or in animal models of asbestosis (Janssen et al., 1995; Janssen et al., 1994b; Mossman et al., 1990). The observations that a number of fibrogenic dusts, such as crystalline silica, caused oxidative damage to cells, spurred many investigations in both cells and rodents confirming that oxidants were mediators of several mineral dust-induced diseases (reviewed in Mossman and Glenn, 2013; Shukla et al., 2003).

5. Links Between Cell Transformation and Epigenetics in Mesothelioma

Knowing that DNA in chromosomes is surrounded by histones and juxtaposed with other proteins, an important conclusion consistent with epigenetic concepts of cancer, is that these proteins are the targets of oxidants elicited by the mesotheliomagenic amphibole asbestos types, crocidolite and amosite. As shown in Fig. 1 below, it is well documented that crocidolite asbestos fibers interact with a number of receptors on the plasma membrane in initial interactions with cells. These interactions lead to activation or inactivation of a number of protein cascades linked to parameters of cell transformation (reviewed in Mossman et al., 2013). At high concentrations of fibers that resulted in human mesotheliomas in the past workplace, one might assume that normal antioxidant defenses were overwhelmed, favoring carcinogenic events. It is likely that many of these events were initiated or perpetuated by a number of protein signaling cascades that have been elicited by crocidolite asbestos fibers in human mesothelial cells (Janssen et al., 1995; Manning et al., 2002; Mossman et al., 2000; Pache et al., 1998; Perderiset et al., 1991; Ramos-Nino et al., 2002; Scapoli et al., 2004; Sesko et al., 1990).

Epigenetic signaling and/or translational modifications of protein may also be critical to asbestos-induced carcinogenesis. This conclusion is supported by number of studies documenting the importance of transcriptomes and protein expression in development of mesotheliomas in animal models and human tissues (Chernova et al., 2017; Christensen et al., 2009; Ramirez-Salazar et al., 2014; Sugarbaker et al., 2008). Most importantly, it has been shown that human mesothelial cell transformation to malignancy is caused by epigenetic modification (Pacaud et al., 2014). In these studies, global DNA hypomethylation of human mesothelial (MET5A) cells caused transformation to malignancy as documented after their injection into immunocompromised mice. Lastly, a number of recent studies point to a number of microRNAs that are altered in expression in human mesothelioma cell lines (reviewed in Robb et al., 2017). Their function in cell transformation and/or invasion has been confirmed in both overexpression and deletion studies.

In conclusion, the past and present concepts of cancer development are presented in Fig. 1 where it is acknowledged that past emphasis on

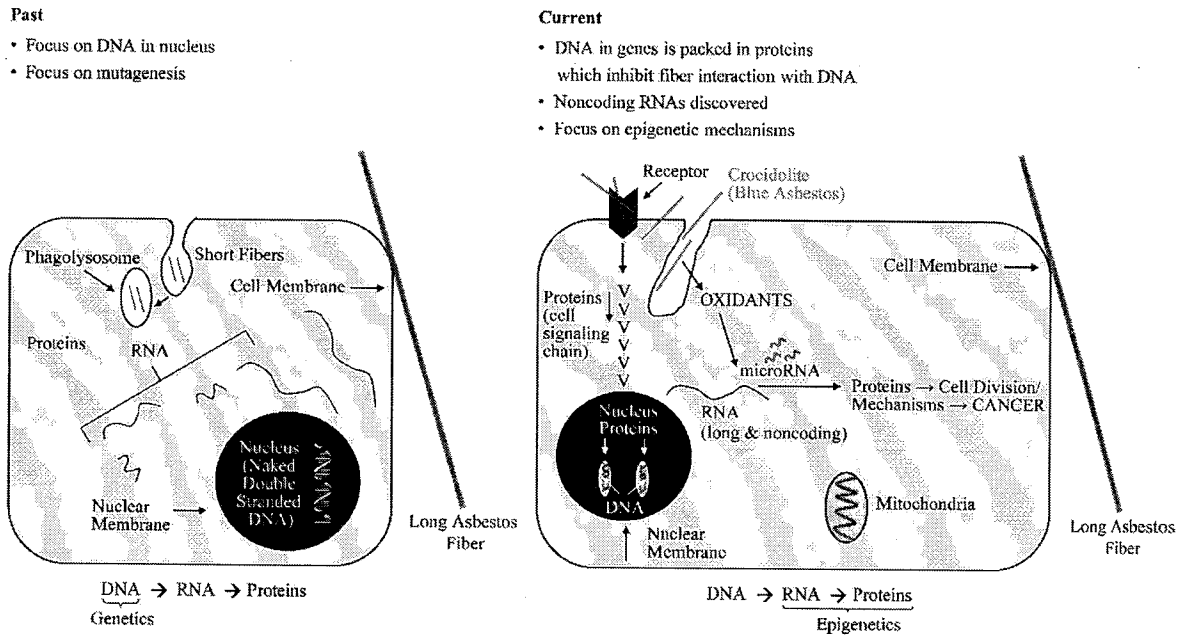

Fig. 1. A comparison between historical and modern concepts of carcinogenesis with analogies to asbestos carcinogenesis. Long (> 5 μm) amphibole fibers that cannot be encompassed by cells remain in the tumor microenvironment where they can act as tumor promoters in the generation of oxidants and cell proliferation. In contrast, short fibers are cleared by cell uptake and other defense mechanisms.

genetic mechanisms has been updated to include the discovery of noncoding RNAs and other epigenetic mechanisms that have been linked to asbestos-induced cancers (reviewed in Mossman, 2017). Cell and organ culture experiments have been crucial in elucidating mechanisms of fiber carcinogenesis and mesothelioma (Singh et al., 2017). Moreover, the importance of fiber length has been demonstrated in rodent models of disease and human tissues (reviewed in Roggli, 2015). Although *in vitro* studies and rodent tumor models do not mimic the differences in fiber type, potency and durability observed in human mesotheliomas because of their shorter time frame, they have elucidated and confirmed the importance of long (> 5 μm) fiber length in a number of carcinogenic and fibrogenic cell responses. For example, studies by Davis and colleagues (Davis et al., 1986; Davis and Jones, 1988) have shown that neither fibrosis nor pulmonary neoplasms appear after inhalation of a short-fiber preparation of amosite. Intraperitoneal injections of short chrysotile produced no mesotheliomas at the lowest concentrations used, suggesting a threshold for short fiber responses. The strengths and limitations of various models of exposure to asbestos in rodents are discussed by our group and others in recent publications (Drummond et al., 2016; Mossman et al., 2011).

Conflict of interest

Dr. Mossman provides consultation for a fee to plaintiffs and defendants in asbestos litigation.

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Exhibit 116

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF NEW JERSEY

- - -

IN RE: JOHNSON & JOHNSON TALCUM POWDER PRODUCTS MARKETING, SALES PRACTICES, AND PRODUCTS LIABILITY LITIGATION	:	NO. 16-2738 (FLW) (LHG)
THIS DOCUMENT RELATES TO ALL CASES	:	

- - -

September 25, 2018

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Videotaped deposition of JOSHUA E. MUSCAT, Ph.D., taken pursuant to notice, was held at the law offices of Drinker Biddle & Reath, One Logan Square, Philadelphia, Pennsylvania, beginning at 9:45 a.m., on the above date, before Michelle L. Gray, a Registered Professional Reporter, Certified Shorthand Reporter, Certified Realtime Reporter, and Notary Public.

- - -

GOLKOW LITIGATION SERVICES
877.370.3377 ph | 917.591.5672 fax
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BY MR. TISI:

Q. Now, you said you became an expert consultant with them in 2010?

A. Yes.

Q. And in fact, you have been identified as an expert in litigation on behalf of Shook Hardy & Bacon, correct?

A. That's correct.

Q. You were paid for that?

A. Yes.

Q. When did that relationship start?

A. Well, approximately 2010.

Q. So it would have started before your 2011 article that wasn't listed on your CV was submitted for peer review?

MR. HEGARTY: Objection to form.

MR. HUDSON: Objection to form.

THE WITNESS: I don't know when that was submitted.

BY MR. TISI:

Q. It was submitted in April of 2011.

A. Okay.

Q. If that were true, you were already working as a consultant, an expert, for Shook Hardy & Bacon, true?

MR. HEGARTY: Objection to form.

THE WITNESS: I don't know. Perhaps.

BY MR. TISI:

Q. That wasn't disclosed on that article, was it?

A. I didn't write the article.

Q. It went in under your name, sir, did it?

A. Yes.

Q. And you're also were aware Dr. Huncharek was retained as an expert by Shook Hardy & Bacon as well?

A. That's correct.

Q. And he didn't disclose -- you were retained at about the same time, true?

Exhibit 117

Use of cosmetic talc on contraceptive diaphragms and risk of ovarian cancer: a meta-analysis of nine observational studies

Michael Huncharek[a,b], Joshua Muscat[c], Adedayo Onitilo[b] and Bruce Kupelnick[a]

Prior work suggests an association between perineal use of cosmetic talc and increased ovarian cancer risk. A meta-analysis was performed to examine this hypothesis by evaluating ovarian cancer risk associated with direct exposure of the female genital tract to talc via dusting of contraceptive diaphragms. Data were pooled from epidemiological studies using a general variance-based meta-analytic method that employs confidence intervals. The outcome of interest was a summary relative risk reflecting the risk of ovarian cancer development associated with the use of cosmetic talc on contraceptive diaphragms. Sensitivity analyses were performed to explain any observed statistical heterogeneity and to explore the influence of specific study characteristics on the summary estimate of effect. Initially, combining homogeneous data from nine case–control studies yielded a non-statistically significant summary relative risk of 1.03 (0.80–1.37), suggesting no association between talc-dusted diaphragms and ovarian cancer development. Sensitivity analyses were performed to evaluate the robustness of this finding. All resultant summary relative risks were not statistically significant. The available epidemiological data do not support a causal association between the use of cosmetic talc-dusted diaphragms and ovarian cancer development. *European Journal of Cancer Prevention* 16:422–429 © 2007 Lippincott Williams & Wilkins.

European Journal of Cancer Prevention 2007, 16:422–429

Keywords: diaphragms, ovarian neoplasms, systematic review, talcum powder

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Introduction

Ovarian cancer represents a major cause of cancer-related morbidity and mortality in the United States with an estimated 22 000 new cases diagnosed in 2005 (Boger-Meigiddo and Weiss, 2005). It is the seventh most common cancer in women and ranks fourth as a cause of cancer deaths among female individuals from the United States, with some 16 000 succumbing to the disease this year. The lethality of ovarian tumors is in large part due to the fact that clinical symptoms tend to occur late in the natural history of the disease and the lack of screening tests allowing for early diagnosis. In fact, approximately 60% of patients are diagnosed with late-stage disease (stage III and IV) vastly diminishing the chance of long-term survival (approximately 10% at 5 years from diagnosis) (Richardson *et al.*, 1985).

Primary prevention of ovarian cancer remains elusive as a clear etiology for the vast majority of cases is unknown. Nonetheless, prior epidemiological research suggests a number of risk factors, including age (older versus younger), nulliparity, first pregnancy after the age of 35 years, diet high in saturated fats, positive family history of ovarian/breast cancer and race (white versus African American) (Baker and Piver, 1994; Tortolero-Luna and Mitchell, 1995; Daly and Obrams, 1998). Clear geographic differences in incidence exist. The highest rates are found in industrialized countries versus underdeveloped nations (Ioka *et al.*, 2003), implicating environmental factors in ovarian cancer etiology. The one exception is highly industrialized Japan (Ioka *et al.*, 2003) with a low annual incidence of approximately 3/100 000. Interestingly, Japanese woman who migrate to the United States experience an increased occurrence of this disease, further suggesting environmental factors in its cause.

In 1982, Cramer *et al.* (1982) published the first study suggesting a link between use of cosmetic talc and the risk of developing ovarian cancer. Subsequently, a number of additional reports have shown a small but increased risk among women using cosmetic talc products, although this finding is not universal (Chang and Risch, 1997). These statistical associations raise concerns that a cause–effect relationship may exist between talc exposure (particularly perineal use) and ovarian carcinogenesis.

Further fueling concerns about this association is the mistaken, but often repeated, assertion that asbestos and talc are biologically similar; that is, they may exhibit similar disease-causing potential (Wong *et al.*, 1999). While talc and asbestos are both silicates, they bear little resemblance structurally or in their biological properties. Asbestos fibers are well recognized human and animal carcinogens with substantial supporting epidemiological and in-vivo evidence available in the published literature (Huncharek, 1986; Mossman and Gee, 1989). Asbestos is known to induce peritoneal (and pleural) mesotheliomas among occupationally and environmentally exposed cohorts and some evidence exists suggesting that asbestos can also cause ovarian neoplasms in humans (Acheson *et al.*, 1982).

Although in the experimental setting translocation of talc particles to the human ovary can occur with deliberate or inadvertent manipulations of patients in the supine position (Wehner, 1998), it is unknown whether cosmetic use of talc in the perineal area can routinely penetrate the female reproductive tract and reach the ovary against physiological forces working in the opposite direction. The existing epidemiological literature focuses primarily on external perineal exposure. It appears, however, that the talc–ovarian cancer hypothesis could be tested with better precision and validity if the exposure to the suspected carcinogen was directly to the reproductive tract. A common route for such an exposure is via talc dusting of contraceptive diaphragms, a well documented practice in the relevant epidemiological literature. Intuitively, the possible association of ovarian cancer with talc-dusted diaphragms appears to provide a more rational test of this cause–effect hypothesis. Therefore, the present report describes the results of a meta-analysis pooling data from nine epidemiological studies examining the risk of ovarian cancer associated with the use of cosmetic talc on diaphragms.

Methods

The methods employed in the design and execution of this analysis have been previously described (Greenland, 1986; Cooper and Hedges, 1994). A study protocol was prospectively developed outlining the purpose and methods; that is, a meta-analysis examining the risk of developing ovarian cancer associated with use of talc-dusted contraceptive diaphragms. Eligibility criteria for study inclusion were determined prospectively as were the specific data elements to be extracted from each published report. The study protocol included details of the planned statistical analysis.

We used a data extraction form designed for recording relevant information from each selected report. Two researchers performed data extraction with differences in extraction forms resolved by consensus. Other data collected but not included in the eligibility criteria were the number of patients in each study, study odds ratios or relative risks, 95% confidence intervals and type of statistical adjustments made, if any, by individual study authors.

Literature search

Information retrieval was performed by previously described methods (Cooper and Hedges, 1994). We conducted a MEDLARS search of the literature published between January 1966 and March 2005, as well as a review of Cancer Lit and the CD-ROM version of Current Contents. The search criteria included all languages. The search terms used were talc exposure and ovarian neoplasms. If a series of articles was published, all data were retrieved from the most recent article. The literature search also included hand searches of bibliographies of published reports, review articles and textbooks.

The initial citations (in the form of abstracts) from this literature search were screened by a physician investigator to exclude those that did not meet inclusion criteria. Reasons for rejection included study designs other than case–control, cohort or randomized controlled trials; animal or in-vivo studies; abstracts; review articles and non-peer reviewed articles. Eligibility criteria included, observational studies or clinical trials enrolling patients with histologically proven ovarian tumors of all histologies, studies enrolling only adult patients (i.e. 18 years or older), availability of data documenting type of talc exposure, in this instance, dusting of diaphragms, and availability of odds ratios or relative risks with 95% confidence intervals for each report or availability of raw data to calculate these parameters.

Statistical analysis

We performed data analysis according to meta-analytic procedures described by Greenland (1986). This method of meta-analysis is a general variance-based method employing confidence intervals. As the variance estimates are based on the adjusted measures of effect, the confidence interval methods do not ignore confounding and are the preferred methodology for pooling observational studies.

For each included study, we derived odds ratios reflecting the risk of developing ovarian cancer associated with the practice of dusting contraceptive diaphragms with cosmetic talc and determined the natural logarithm of the estimated relative risk for each data set followed by calculation of an estimate of the variance. We used the estimate of the 95% confidence interval from each study to calculate the variance of each study's measure of effect.

We calculated a weight for each included analysis as 1/variance followed by a summation of the weights. We then determined the product of the study weight and the natural logarithm of the estimated relative risk and performed a summation of these products. Finally, a summary relative risk and 95% confidence interval were determined.

Before the estimation of a summary relative risk, a statistical test for homogeneity was performed (Q). This procedure tests the hypothesis that the effect sizes are equal in all of the included studies (Greenland, 1986). If Q exceeds the upper tail critical value of χ^2 ($P < 0.10$) at $k-1$ d.f. (where k equals the number of studies analyzed or the number of comparisons made), the observed variance in study effect sizes is significantly greater than what would be expected by chance if all studies shared a common population effect size. If the hypothesis that the studies are homogenous is rejected, the studies do not measure an effect of the same size. In this instance, calculation of a pooled estimate of effect (i.e. relative risks) may be of questionable validity. Possible explanations for the observed heterogeneity must be sought to provide the most rational interpretation of the summary relative risk. Sensitivity analyses and or further stratified analyses are then performed based on the magnitude of Q.

Results

The literature search yielded 17 studies that appeared to meet protocol specifications and full papers were obtained for review (Hartge *et al.*, 1983; Richardson *et al.*, 1985; Whittemore *et al.*, 1988; Booth *et al.*, 1989; Harlow and Weiss, 1989; Chen *et al.*, 1992; Harlow *et al.*, 1992; Rosenblatt *et al.*, 1992; Tzonou *et al.*, 1993; Purdie *et al.*, 1995; Cook *et al.*, 1997; Goddard *et al.*, 1998; Cramer *et al.*, 1999; Gertig *et al.*, 2000; Ness *et al.*, 2000). Upon further review, nine of these met the specified inclusion criteria. Table 1 provides an overview of the nine reports included in the meta-analysis (Hartge *et al.*, 1983; Richardson *et al.*, 1985; Whittemore *et al.*, 1988; Booth *et al.*, 1989; Harlow and Weiss, 1989; Harlow *et al.*, 1992; Rosenblatt *et al.*, 1992; Cook *et al.*, 1997; Ness *et al.*, 2000). A total of 2281 ovarian cancer cases and 3608 controls were enrolled in nine case–control studies. Table 1 also specifies which reports were hospital based versus those that were population based. Only Cook *et al.* (1997) and Harlow and Weiss (1989) used both population-derived cases and controls. All of the other studies listed as 'population based' used hospital-derived cases. The individual study odds ratios listed in Table 1 reflect the odds of exposure in cases versus controls, with an odds ratio greater than one suggesting a positive association, that is, an increased risk of ovarian cancer among women using talc-dusted diaphragms.

Before combining all studies to derive a summary estimate of effect (i.e. a summary relative risk) a statistical test for heterogeneity was performed (Q). This gave a value of Q equal to 10.75. With eight degrees of freedom, the P value associated with a Q of this size is 0.22. This indicates that the studies are homogeneous; that is, the studies are measuring an effect of similar

Table 1 Overview of included studies

Study (year)	Number of cases/controls	Percentage eligible cases included	Adjusted OR	95% CI	Adjustments to OR	Epithelial tumors only	Borderline tumors incl.	Stratification by histology	H/P
Booth *et al.* (1989)	235/451	84	0.75	0.85–2.02	Age, SES	Y	Y	N	H
Cook *et al.* (1997)	313/422	64	0.80	0.40–1.40	Age	Y	N+	Y	P
Cramer *et al.* (1982)	215/215	72	1.56	0.62–3.88	Parity, menstrual status	Y	Y	Y	P
Harlow *et al.* (1992)	235/239	59	1.20	0.60–2.40	Parity, education, marital status, religion, use of sanitary napkins, douching, age, weight	Y	Y	Y	P
Harlow and Weiss, 1989	116/158	68	0.50	0.20–1.30	Age, parity, use of oral contraceptives	N/A	All	N/A	P
Hartge *et al.* (1983)	135/171	69	0.80	0.40–1.40	Age, race, hospital	Y	Unknown	N	H
Ness *et al.* (2000)	767/1367	61	0.60	0.30–1.20	Age, gravity, race family HX ovarian cancer, oral contraceptive use, tubal ligation, hysterectomy, breast feeding	Y	Y	N	P
Rosenblatt *et al.* (1992)	77/46	55	3.0	0.80–10.8	Obesity, SES, religion, number of live births, OC use	Y	Unknown	N	H
Whittemore *et al.* (1988)	188/539	NG	1.5	0.63–3.58	Parity, use of oral contraceptives	Y	Unknown	N	H

SES, socio-economic status; OR, odds ratio; CI, confidence interval; H/P, hospital based/population based; N+, separate analyses done for borderline versus invasive tumors.

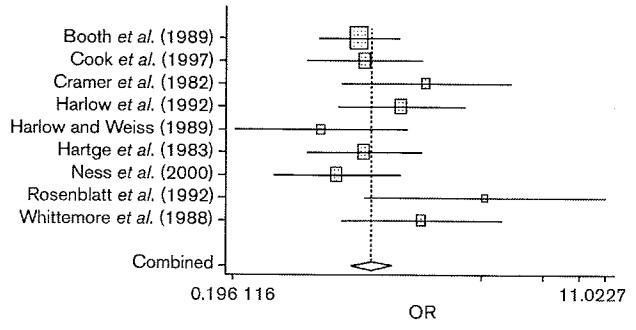
magnitudes. Given the lack of statistical heterogeneity, the data were pooled for calculation of a summary relative risk.

Table 1 shows that adjusted odds ratios ranged from 0.60 (Booth *et al.*, 1989) to 3.0 (Rosenblatt *et al.*, 1992), with adjustment parameters specified along with 95% confidence intervals. Of note, none of the reports showed a statistically significant odds ratio. Initial pooling of data from all nine reports yielded a summary relative risk of 1.03 with a 95% confidence interval of 0.80–1.33, a non-statistically significant result suggesting no association between talc/diaphragm use and ovarian cancer risk (see Fig. 1).

Upon closer scrutiny of the available data, further sensitivity analyses were performed as described below. The data provided by Booth *et al.* (1989) did not explicitly provide data on talc use via contraceptive diaphragms and such use could only be assumed. As the data were questionable in this respect they were dropped from the analysis and a summary relative risk was recalculated. The resultant relative risks was 1.12 with a 95% confidence interval of 0.84–1.48. Therefore, the results remained statistically non-significant despite removal of these data from the summary estimate of effect.

The report by Harlow *et al.* (1992) also represents a potential problem for statistical pooling as the cases in this instance were all patients with 'borderline ovarian tumors'. The exact nature of borderline ovarian tumors in terms of a relationship with their invasive counterparts remains unclear, with some data suggesting differences in epidemiology and etiology (Riman *et al.*, 2001). Whether borderline tumors are precursors of invasive cancers or a separate disease entity is a matter of debate. We therefore recalculated a summary relative risk without inclusion of data from the study by Ness *et al.* (2000). This gave a relative risk of 1.09 with a 95% confidence interval of (0.84–1.41), a non-statistically significant result.

Fig. 1



Forest plot of summary relative risk derived by pooling all available studies using adjusted odds ratios (OR).

All studies except that of Hartge *et al.* (1983) are full research reports with the study by Ness *et al.* (2000) published as a 'Letter to the editor'. Publication in this format is potentially problematic owing to issues related to the 'quality' of the presented data. Letters to the editor normally do not undergo the same type of editorial scrutiny as full research papers. In addition, by their nature, letters are brief notes with limited details presented, precluding rigorous evaluation of methods, results and associated conclusions. In order to address these issues, we dropped the study by Hartge *et al.* from the pooled analysis and, again, recalculated a summary relative risk. This gave a relative risk of 1.07 with a 95% confidence interval of 0.82–1.40. Again, this represents a non-significant finding.

In a prior meta-analysis (Huncharek *et al.*, 2003), we demonstrated a possible bias among studies examining the perineal talc use/ovarian cancer association based on the source of cases. That is, our study suggested that population-based studies may spuriously show a causal association secondary to exposure misclassification to a 'treatment effect' among population-derived cases. Some patients with ovarian cancer will undergo treatment with radiation, chemotherapy and/or surgery. Side effects from treatment may prompt talc use among some of these individuals. Patients may not always make the distinction between pre-diagnosis and post-treatment use. Exposure misclassification among 'prevalent' cases may cause a spurious finding of an association when none, in fact, exists. We therefore recalculated the summary relative risk excluding the studies by Cook *et al.* (1997) and Harlow and Weiss (1989) as these were the only two reports that utilized population-derived cases and controls. The resultant relative risk was 1.15 with a non-statistically significant odds ratio of 0.87–1.53.

Furthermore, this suggests no association between talc use and increased ovarian cancer risk. In fact, if data from the studies by Cook *et al.* (1997) and Harlow and Weiss (1989) are statistically pooled, the summary relative risk is 0.67 with a non-significant confidence interval (i.e. 0.34–1.35). The fact that the population-based relative risk is in the opposite direction (i.e. favoring a protective effect for talc) to that shown in the other case–control studies, further supports the existence of bias in these analyses.

Another methodological consideration is the fact that the definitions of the control groups used across all nine studies are not completely comparable. Some reports defined controls as 'never having used talc' (e.g. Ness *et al.*, 2000), while others used controls defined as not

having used talc on diaphragms (e.g. Cook *et al.*, 1997). We therefore calculated crude odds ratios and 95% confidence intervals using data supplied in the available studies and recalculated a summary relative risk to ensure that the analysis using adjusted odds ratio was not spurious (Table 2). The resultant relative risk was 0.86 (0.59–1.40) (see Fig. 2), a non-statistically significant result suggesting no association between talc use on diaphragms and increased ovarian cancer risk (see Fig. 2). Of note, the test for heterogeneity for this latter analysis gave a value for Q of 7.20 with a P value of 0.52.

Discussion

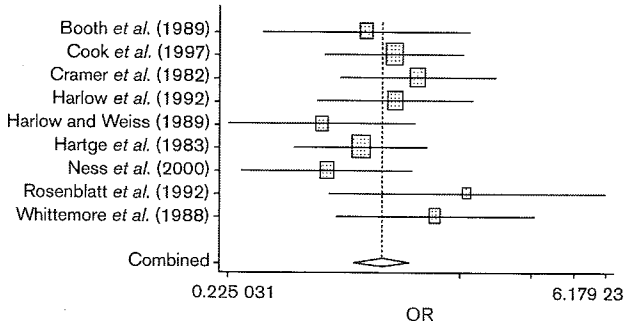
Talc is an important industrial mineral for a number of reasons including its resistance to heat, electricity and acids and its relatively low price. It is used in many commercial applications because of its lamellar platy nature, softness, whiteness, chemical inertness, high melting point and hydrophobic features, among others. For instance, talc is used in the plastic industry owing to its inertness, superior electrical and thermal resistance and its ability to improve the quality of plastic surfaces. It also finds application in the paint industry to increase the smoothness of paint products and in paper manufacturing to reduce the usage of expensive whitening agents because of its high brightness.

Table 2 Crude odds ratios and 95% confidence intervals for included studies

Study (year)	Crude OR	95% CI	Variance	Weight
Booth *et al.* (1989)	0.75	0.33–2.02	0.175	5.70
Cook *et al.* (1997)	0.96	0.52–1.76	0.097	10.2
Cramer *et al.* (1982)	1.18	0.59–2.35	0.125	7.99
Harlow *et al.* (1992)	0.97	0.49–1.92	0.121	8.24
Harlow and Weiss, 1989	0.51	0.22–1.13	0.184	5.43
Hartge *et al.* (1983)	0.72	0.40–1.30	0.090	11.1
Ness *et al.* (2000)	0.53	0.25–1.13	0.147	6.80
Rosenblatt *et al.* (1992)	1.82	0.55–6.34	0.373	2.68
Whittemore *et al.* (1988)	1.38	0.57–3.28	0.204	4.91

OR, odds ratio; CI, confidence interval.

Fig. 2



Forest plot of summary relative risk derived by pooling all available studies using crude odds ratios (OR).

Mineral talc is a magnesium silicate hydroxide belonging to the mineral class, silicate and subclass phyllosilicate. It belongs to the clay mineral group, an important subgroup within the phyllosilicates that contain large percentages of water trapped between the silicate sheets. Clay minerals are divided into four major groups: the kaolinite group, the montmorillonite/smectite group, the illite group and the chlorite group. Talc is a member of the montmorillon/smectite group along with pyrophyllite, vermiculite, sauconite, saponite and nontronite.

Talc also forms pseudomorphs, that is false shapes, of other minerals, replacing them on an atom by atom basis. For instance, talc forms pseudomorphs of quartz, pyroxene, olivine and amphiboles. In nature, it can also be found in association with a number of other minerals, such as serpentine, quartz, olivine and biotite.

In 1982, Cramer *et al.* (1982) published a case–control study suggesting an association between cosmetic talc use on the perineum and increased ovarian cancer risk. Women dusting the perineum with talc or dusting sanitary napkins showed a near doubling of ovarian cancer risk. Unfortunately, in addition to a number of methodological limitations plaguing this report (e.g. only 45% of eligible controls participating), it is important to point out the flawed premise on which it is based. Cramer *et al.* (1982) cite the 'chemical relationship between talc and asbestos' as a major reason for assuming that talc may also be a human carcinogen and that '...the mineral talc is a specific hydrous magnesium silicate chemically related to several asbestos group minerals and occurring in nature with them'.

The above-cited justification for the Cramer *et al.* (1982) study and subsequent work examining a possible cosmetic talc/ovarian cancer link is misguided for a number of reasons. Despite the fact that talc and various forms of asbestos are silicates, they are structurally distinct and belong to different mineral groups and subgroups. That is, amphibole minerals (e.g. tremolite) are inosilicates while talc is a member of the silicate subclass phyllosilicate and the group, clay or montmorillonite/smectite. While serpentines, including serpentine asbestos, are also phyllosilicates, serpentine minerals belong to the kalolinite–serpentine group. The asbestos varieties of serpentine are structurally different from other members of the serpentines in that their brucite layers and silicate layers bend into tubes that produce fibers. Non-fibrous serpentine does not have carcinogenic properties and it is clear that the physical structure of serpentine asbestos is responsible for its disease-causing

potential, not its atomic constituents. It simply does not follow, therefore, that one should assume that talc is carcinogenic simply because it is a silicate and a member of the phyllosilicate subgroup. Structure dictates toxicity/carcinogenicity, not chemical composition.

It is true that in nature, mineral talc can be found in association with both serpentine and amphibole minerals, including the asbestos varieties. It is crucial to understand that the carcinogenic potential of asbestos is well known and abundantly documented in the medical and epidemiological literature (Huncharek, 1986; Mossman and Gee, 1989). Cramer *et al.*'s argument suggesting that pure talc is carcinogenic is based solely on 'guilt by association' rather than on scientific fact. If one is exposed to a mixture of talc and asbestos, it is reasonable to expect a carcinogenic effect as it contains a known carcinogen. To then suggest that talc is also carcinogenic simply owing to the fact that it is sometimes found in association with various asbestos minerals in nature is not logical. This reasoning ignores a large body of data regarding the mineralogy of silicates and fails to acknowledge the lack of supporting biological or in-vitro data documenting any carcinogenic potential of pure talc (i.e. uncontaminated by asbestos). A commercial product containing asbestos-contaminated talc could certainly pose a health risk and although prior to the mid-1970s some consumer talc products did, in fact, contain such contamination, the carcinogenic entity is asbestos, not talc (Rohl *et al.*, 1976). It is important to note that since that time, talc product manufacturers voluntarily ensured that such products are asbestos free. Despite this fact, even some recent studies looking at the perineal talc dusting/ovarian cancer risk connection show a weak association (e.g. Mills *et al.*, 2004), further suggesting a spurious finding.

Other evidence that indicates that talc and asbestos have dissimilar biological properties is the fact that talc has been used for decades as a sclerosing agent for both benign and malignant pleural effusions (Viskum *et al.*, 1989). Long-term follow-up studies of these patients have not shown even a single case of lung cancer or mesothelioma resulting from introduction of talc to the pleural cavity (Viskum *et al.*, 1989; Shaw and Agarwal, 2004). Epidemiological studies of talc miners and millers also fail to demonstrate an increased cancer risk (Rubino *et al.*, 1976; Gamble, 1993). In-vivo implantation and injection using asbestos of various types, in contrast, unequivocally induce tumors in experimental animals (Huncharek, 1986).

Despite the above-noted problems, the idea that cosmetic talc poses a possible ovarian cancer risk persists. As reviewed in the present paper and elsewhere (Richardson *et al.*, 1985; Tortolero-Luna and Mitchell, 1995) numerous investigators have examined this possible relationship in a variety of case–control studies and at least one cohort study (e.g. Gertig *et al.*, 2000). Most of these categorized talc use as 'ever versus never' used while others further stratified by particular types of use, for example, perineal dusting, sanitary napkin dusting, condoms, etc. Results differ across studies, with some showing no association (Rosenblatt *et al.*, 1992) while others suggests a 'weak effect' (Purdie *et al.*, 1995), that is odds ratios below 1.5.

In addition to the obvious problems with the premise put forth by Cramer *et al.* (1982) and others, validity of the weak effect shown in a number of other epidemiological studies also remains questionable. The major weaknesses of the existing database include (Boger-Meigiddo and Weiss, 2005) the relatively small sample size of most reports, which limits the statistical power to detect an effect (Richardson *et al.*, 1985), the lack of consistent positive association across studies (Baker and Piver, 1994), the absence of a demonstrable dose–response relationship (Daly and Obrams, 1998), the lack of supporting evidence of talc carcinogenicity from animal or in-vitro analyses (Tortolero-Luna and Mitchell, 1995) and the possible presence of uncontrolled confounding producing a spurious positive association. In fact, some of the available observational studies show an inverse dose–response (Ness *et al.*, 2000) that weighs against a causal association. In addition, no plausible biological mechanism capable of explaining how talc could induce ovarian malignancies exists.

In a study, Heller *et al.* (1996) examined talc particle counts in ovarian specimens from 24 women undergoing incidental oophorectomy and compared these counts with reported frequency and duration of talc use. The study sought to examine the hypothesis of a dose-related risk of epithelial ovarian cancer with perineal talc exposure. Women were considered 'exposed' if they reported talc application to undergarments or directly to the perineum. Talc was detected in all ovaries by either polarized light or electron microscopy. No relationship was found between cosmetic talc burden in healthy ovarian tissue and lifelong perineal talc dusting determined by either microscopic methods. This study raises further questions regarding whether reported associations between perineal talc exposure and ovarian tumors in observational studies reflects a carcinogenic action of talc. The validity of these epidemiologic associations has also been questioned because it is unknown whether talc dust in the perineal area can actually penetrate the female reproductive tract and then translocate to the ovaries against physiological forces working in the opposite direction. The work of Heller *et al.* clearly brings this into question.

Although the epidemiological literature focuses primarily on external perineal exposure to talc, a more valid

assessment of the 'talc hypothesis' would appear to be provided by examining the ovarian cancer risk associated with talc dusting of diaphragms. This particular use of talc results in direct female reproductive tract exposure. Although data on the use of talc-dusted diaphragms have been reported in some epidemiological studies, this literature fails to garner the attention devoted to perineal dusting and no systematic evaluation of this particular literature is available. This probably reflects the fact that perineal dusting is a more common practice than dusting contraceptive diaphragms. Nonetheless, exposure via this latter route is, intuitively, a better 'model' for testing whether talc represents a risk factor for ovarian cancer as the exposure is directly to the female genital tract. Consequently, we performed the above-detailed meta-analysis pooling all available published data on this topic.

Using accepted meta-analytic techniques our analysis was unable to demonstrate any increased risk of ovarian cancer associated with use of talc-dusted diaphragms. Despite performing a number of sensitivity analyses to test the robustness of our findings, the pooled data from over 5000 cases and controls failed to show a positive association. In some studies, the odds ratio was calculated based on an inappropriate control group; for example, individuals who reported no exposure to any talc. For these studies, the crude odds ratio was recalculated based on women who never used talc-dusted diaphragms as the reference group. This summary relative risk was also statistically non-significant.

In summary, our present report, along with our prior meta-analysis pooling data from studies examining the possible ovarian cancer risk associated with perineal talc dusting (Huncharek *et al.*, 2003), does not provide evidence of a causal relationship. In the context of 'weak associations', many sources of bias and uncontrolled confounding can contribute to the finding of a spurious association. Recall bias in case–control studies, lack of a demonstrated dose–response in many published analyses, lack of a coherent biological mechanism for possible talc carcinogenicity and lack of supporting animal or in-vitro data demonstrating the carcinogenic potential of talc all argue against a causal relationship. These limitations and inconsistencies have also been discussed in detail elsewhere (Wehner, 1994; Muscat and Barish, 1998). As ovarian cancer remains a major cause of cancer-related morbidity and mortality in the United States, further work is needed to clearly define modifiable risk factors in an attempt to improve disease prevention.

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Exhibit 118

Perineal talc use and ovarian cancer risk: a case study of scientific standards in environmental epidemiology

Michael Huncharek[a] and Joshua Muscat[b]

A number of observational studies (largely case–control) conducted over the last two decades suggest an association between use of talc powders on the female perineum and increased risk of ovarian cancer. A subset of these reports shows a roughly 30–60% increased risk of ovarian cancer associated with perineal talc exposure. A number of researchers partly base their conclusions of an association on the '…chemical relationship between talc and asbestos', the latter substance being a known human carcinogen. Although separating causal from noncausal explanations for an observed statistical association is a difficult process, there currently exist commonly accepted guidelines by which such inferences can be made. These scientific approaches include consideration of the strength of the association, the consistency of the finding across studies, and existence of a biological explanation of the observed phenomenon, among others. When applied to the context of a proposed talc/ovarian cancer association, we conclude that the weak statistical associations observed in a number of epidemiological studies do not support a causal association. *European Journal of Cancer Prevention* **20:501–507 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: causation, cosmetic talc, ovarian neoplasms, risk factors

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Introduction

Ovarian cancer represents a major cause of cancer-related morbidity and mortality in the United States, with an estimated 22 000 new cases diagnosed in 2005 (Boger-Megiddo and Weiss, 2005). It is the seventh most common cancer in women and ranks fourth as a cause of cancer deaths among females from the United States, with some 16 000 succumbing to the disease this year. The lethality of ovarian tumors is in large part because of the fact that clinical symptoms tend to occur late in the natural history of the disease and the lack of screening tests allowing for early diagnosis. In fact, approximately 60% of patients are diagnosed with late-stage disease (stages III and IV), vastly diminishing the chance of long-term survival [approximately 10% at 5 years from diagnosis (Richardson *et al.*, 1985)].

Primary prevention of ovarian cancer remains elusive as a clear etiology for the vast majority of cases is unknown. In 1982, Cramer *et al.* published the first study suggesting a link between use of cosmetic talc and the risk of developing ovarian cancer. Subsequently, a number of additional reports have shown a small but increased risk among women using cosmetic talc products, although this finding is not universal (Chang and Risch, 1997). These statistical associations raise concerns that a cause–effect relationship may exist between talc exposure (particularly perineal use) and ovarian carcinogenesis.

On 13 May 2008, Samuel Epstein, MD, Chairman of the Cancer Prevention Coalition, submitted a Citizen's Petition to the Commissioner of the Food and Drug Administration seeking placement of cancer warning labels on talc products. The Petition requests the Commissioner of Food and Drugs to require that all talc products bear labels with a warning such as, 'Frequent application of talcum powder in the female genital area substantially increases the risk of ovarian cancer' (Epstein, 2008).

The claim refers to the first observational study (case–control) suggesting an association between the use of talc powders on the female perineum (by direct dusting or dusting sanitary napkins) and increased risk of ovarian cancer, published in 1982. In this document, the researchers partly base their conclusions of an association on the "…chemical relationship between talc and asbestos", the latter substance being a known human carcinogen. The claim also references a number of additional epidemiological studies conducted after 1982 that have shown a statistical link between talc dusting and ovarian cancer risk. A subset of these reports show a roughly 30–60% increased risk of ovarian cancer associated with perineal talc exposure.

The issues articulated by Epstein *et al.* in relation to the possible carcinogenicity of talc are not uncommon when dealing with interpretation of results derived from observational studies. In a study published 20 years ago, Feinstein provided an insightful and cogent explanation for the myriad problems that plague the process of causal inference as it applies to nonexperimental data (Feinstein, 1988). As he

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points out, most people learn about science by studying experimental methods. These methods largely include direct intervention by the experimenter on whatever entity is under study, whether it be an animal species such as rats or mice, specific chemical compounds, subatomic particles, etc. The scientist, in this context, directly manipulates the study subject/object using established principles of experimental science. In the context of human studies, the experimental design that has come to represent the 'gold standard' of cause–effect relationships is the randomized clinical trial. Unfortunately, in epidemiological research, issues of feasibility and ethical considerations preclude randomization of healthy human participants to receive potentially harmful exposures to various substances, including those that represent possible carcinogenic hazards. Therefore, the epidemiologist must substitute observational methods to study cause–effect relationships that preclude direct intervention with, and/or manipulation of, study participants (i.e. experiments). Owing to this fact, criteria for establishing cause–effect relationships are inherently different when using epidemiological methods versus experimental ones.

In 1965, Hill published a landmark study articulating standards for drawing causal inferences from observational data. His rationale for this stemmed from the realization that the urgency of many public health problems demands action despite the fact that existing knowledge might be imperfect (Rothman, 1986). The 'Hill Criteria' as they have become known, are not simply a 'checklist' of requirements that must be met in order to determine cause–effect relationships. Rather, they represent a theoretical framework to guide one's thinking when attempting to decide whether a body of data meets a basic threshold necessary to distinguish causal from noncausal associations. These criteria include: (i) strength of association, (ii) consistency (i.e. repeated observation of an association in different populations under different circumstances), (iii) specificity (a given cause leads to a specific effect), (iv) temporality (cause must precede effect), (v) biological gradient (dose–response), (vi) plausibility (biological plausibility), (vii) coherence (i.e. that a given cause–effect relationship for an association does not conflict with what is known of the natural history and biology of the disease in question), (viii) experimental evidence (to support the observational findings), and (ix) analogy.

Although the Hill criteria do not provide a complete solution to the dilemma of causal inference in epidemiology, their importance lies in establishing at least a general framework for the process. The proposed talc/ovarian cancer association represents an illustrative example of the utility of this framework. Below we discuss the points raised by Epstein *et al.* in this context and show that the conclusion that the proposed talc/ovarian cancer association is causal is not supported by existing data.

Talc and ovarian cancer: overview of the scientific evidence

The possibility that perineal talc exposure could be associated with development of ovarian cancer was initially derived from a case–control study published in 1982 (Cramer *et al.*, 1982). Since that time, a number of additional reports have addressed this question, with most showing odds ratios (OR) ranging between 1.0 and 2.0 (Table 1). Although this has prompted some to suggest that these estimates of effect provide support for a cause–effect relationship between this exposure and disease outcome, several important caveats must be considered.

Effects of this magnitude are often characterized as 'weak effects' and although the exact definition of a weak effect is debatable, most epidemiologists would consider associations of less than 2.0 to fall within this general category. Hill and others argue that strong associations are more likely to be causal than weak associations as, '...if they were due to confounding or some other bias, the biasing association would have to be even stronger and would therefore presumably be evident' (Rothman, 1986). As Rothman points out, weak associations are more likely to be explained by undetected biases.

Measures of association of this magnitude are often difficult to interpret. This is based on the fact that the investigator cannot directly manipulate the levels of exposure of interest or extraneous factors that could affect study findings. Attempts to control for external factors are accomplished by statistical manipulations of collected data. However, this process depends on the accuracy and completeness of data collection. Further, the correct choice and interpretation of both statistical models and statistical findings can also be contentious.

It is important to point out that although an association is weak, this does not rule out a causal connection. Nonetheless an example of a factor that could confound the weak effect shown for perineal talc is smoking. It is now recognized that smoking is a risk factor for a number of solid tumors including lung cancer (with ORs on the order of 5.0 vs nonsmokers) and esophageal cancer. Evidence exists that smoking may also be related to at least some types of ovarian tumor, in particular those of the mucinous histology (Huncharek *et al.*, in press). The current literature contains a number of reports showing a doubling or tripling of mucinous ovarian cancer risk among smokers (Green *et al.*, 1997; Pan *et al.*, 2004). Interestingly, in a recent meta-analysis of observational studies, Huncharek *et al.* (in press) show that smoking not only increases the risk of mucinous ovarian tumors, but also the more common serous tumors (Table 2). As Rosenblatt *et al.* (1998) reported that smokers are more likely to engage in perineal talc dusting compared with nonsmokers, an imbalance in smokers across case and control groups in epidemiological studies of the talc/ovarian cancer association could contribute to a spurious positive association.

Table 1 Overview of observational studies examining perineal talc use/ovarian cancer risk

Reference	Number of cases	Number of controls	Frequency of powder use	OR (95% CI)	Hospital vs. population based study
Booth *et al.* (1989)	235	451	Never vs. ever	1.29 (0.92–1.80)	H
Chang and Risch (1997)	450	564	None vs. any	1.42 (1.08–1.86)	P
Chen *et al.* (1992)	112	224	Never vs. ever	3.9 (0.9–10.6)	P
Cook *et al.* (1997)	313	422	None vs. any	1.5 (1.1–2.0)	P
Cramer *et al.* (1999)	563	523	Never vs. any	1.60 (1.18–2.15)	P
Cramer *et al.* (2005)	215	215	None vs. any	1.92 (1.27–2.89)	P
Gertig *et al.* (2000)[a]	307		Never vs. ever	1.05 (0.84–1.32)	P
Godard *et al.* (1998)	170	170	Never vs. ever	2.49 (0.94–6.58)	P
Harlow *et al.* (1992)	235	239	Never vs. any	1.5 (1.0–2.1)	P
Harlow *et al.* (1992)	116	158	None vs. any	1.1 (0.7–2.1)	P
Ness and Cottreau (1999)	767	158	None vs. any	1.5 (1.1–2.0)	P
Purdie *et al.* (1995)	824	860	Never vs. ever	1.27 (1.04–1.54)	P
Rosenblatt *et al.* (1998)	77	46	Never vs. any	1.0 (0.2–4.0)	H
Tzonou *et al.* (1993)	189	200	Never vs. any	1.05 (0.28–3.98)	H
Whittemore *et al.* (1998)	188	539	Never vs. ever	1.45 (0.81–2.60)	H
Wong *et al.* (1999)	499	755	Never vs. ever	1.0 (0.8–1.3)	H

CI, confidence interval; H, hospital-based study; OR, odds ratio; P, population-based study.
[a]Cohort study.

Table 2 Summary of meta-analysis results

Risk category	Number of studies	RRs	Statistically homogeneous?
Current/ever smoker	Three cohorts	1.14 (0.93–1.35)	Yes
Current/ever smoker	20 case–control	1.06 (1.01–1.12)	No
Highest vs. lowest pk/years	10 studies (three cohort, seven case–control)	1.21 (1.10–1.31)	No
As above, excluding three studies that combined both borderline and invasive tumors	Seven studies total	1.11 (1.00–1.22)	Yes
Analysis stratified by tumor histology			
Serous tumors			
Current/ever smoker	Four studies	1.28 (0.95–1.61)	Yes
Serous/nonmucinous/other histologies			
Current/ever smoker	Six studies	1.31 (1.15–1.47)	Yes
Mucinous tumors			
Current/ever smoker	Six studies	2.58 (2.23–2.93)	Yes

Pk/years, packs smoked per year; RRs, summary relative risk.

Consistency of an effect could contribute to a causal claim despite a finding of a weak association. Epstein *et al.* characterize the talc/ovarian cancer relationship as being 'confirmed' by multiple scientific publications as well as by review of available evidence by the International Agency for Research on Cancer. They state that, '…International Agency for Research on Cancer concluded that eight publications confirmed a 30–60% increased risk of ovarian cancer following the perineal application of talc'. Despite the claims of the petitioners, a review of available evidence shows that the epidemiological evidence is not consistent across studies or across study types. For instance, Table 1 shows several inconsistencies in the database. Clearly, not all studies showed a positive, statistically significant association, even among the case–control studies that make up the bulk of the database. In addition, there was relatively wide variation in the magnitude of measures of association.

Interestingly, up to the date of filing of the petition by Epstein *et al.*, only one cohort study had been published, that of Gertig *et al.* (2000) that showed no association between perineal talc use and ovarian cancer risk. Given the conflicting findings of case–control studies, Huncharek *et al.* (2003) used meta-analytic techniques to explore possible sources of variability among these reports. Their rationale for doing so was that if meta-analyses showed that the patterns of low relative risks or ODs are consistent across all relevant studies in different populations, these weak associations are less likely to be due to confounding or other biases. If a statistical test for heterogeneity shows effects of different magnitudes across studies, sensitivity analyses can be employed to determine the source of observed variability and thereby identify biases due to study design, case–control selection, etc.

Huncharek *et al.* initially pooled data from 15 case–control and one cohort analysis, yielding a summary relative risk (RRs) of 1.33 (1.16–1.45). Although this suggests a statistically significant positive association between perineal talc use and ovarian cancer risk, sensitivity analyses demonstrated clear differences in outcome based on study

design. That is, hospital-based case–control studies showed no evidence of an effect [1.19 (0.99–1.41)] in contrast to those reports using population-derived controls [1.38 (1.25–1.52)]. More frequent talc use among hospital-based control participants versus population-derived controls does not explain this finding, as the proportion of controls using talc was the same in both groups, that is, 32%. Other factors account for this difference in outcome. These findings suggest bias and bring the validity of the initial pooled RRs into question. The Huncharek report provides some possible explanation for the observed differences and indicates that study outcomes are not consistent. It is possible that the potentially spurious positive association between talc use and ovarian cancer risk is the existence of a 'treatment effect' among cases. Particularly among population-based studies, a varying proportion of cases will be prevalent rather than incident. Some patients with ovarian cancer will undergo treatment with radiation, chemotherapy, and/or surgery. Side effects from treatment may prompt talc use among some patients. Although many questionnaires used in case–control studies may specify talc use before diagnosis, patients may not always make the distinction between prediagnosis and posttreatment use. Exposure misclassification among 'prevalent' cases may cause a spurious finding of an association when none, in fact, exists.

Further supporting the findings of this meta-analysis are the more recent and updated pooled data provided by Langseth *et al.* (2008) cited by the Epstein petition. These researchers pooled data from 20 relevant epidemiological studies. Again, although the calculated summary RR obtained from pooling data from all 20 reports gives a statistically significant RRs (pooled odds ratio) of 1.35 (1.26–1.46), the statistical test for data heterogeneity yielded a P value of 0.036. A P value of this size (i.e. <0.10) is indicative of significant heterogeneity and, as per convention (Petitti, 2000), precludes statistical pooling, that is the pooled summary estimate of effect is not valid given that the data are heterogeneous. This shows that the available data are not consistent and therefore makes a causal association less likely.

One of the more persistent findings among the epidemiological studies examining this suspected association is the lack of a dose–response relationship. Table 3, derived from data presented in the meta-analysis by Huncharek *et al.*, displays dose–response data for those included studies providing such information. Many of the reports do not show increased risk with increasing exposure. The even more problematic finding in terms of establishing a causal association is that a number of studies suggest that risk decreases with increased exposure (Huncharek *et al.*, 2003).

Few researchers directly address the above-noted lack of evidence of a dose–response relationship. Huncharek *et al.* (2003) and Huncharek and Muscat (2007), in contrast, offer a number of possible explanations for an inverse

Table 3 Talc dose–response data for perineal application and ovarian cancer risk

Reference	Years of talc use/ OR+95% CI	Number of talc applications per month/OR+95% CI
Booth *et al.* (1989)	NG	1 0.7 (0.3–1.8)
		4 2.0 (1.3–3.4)
		30 1.3 (0.8–1.9)
Chang and Risch (1997)	<30 1.7 (1.09–2.68)	<10 1.84 (1.24–2.73)
	30–40 1.44 (0.96–2.15)	10–25 1.13 (0.74–1.72)
	>40 0.96 (0.54–1.38)	>25 0.95 (0.61–1.49)
Cook *et al.* (1997)	0–5.5 1.8 (0.9–3.5)	NG
	5.5–13.5 1.6 (0.9–2.9)	NG
	13.5–27 1.2 (0.6–3.4)	NG
	>27 1.8 (0.9–3.4)	NG
Cramer *et al.* (1999)	<20 1.9 (1.2–3.0)	<30 2.2 (1.4–3.6)
	20–30 1.3 (0.8–2.3)	30–39 1.2 (0.81.8)
	>30 1.4 (0.9–2.3)	40+1.6 (0.8–3.1)
Gertig *et al.* (2000)	NG	4–24 0.99 (0.67–1.46)
		≥ 30 1.12 (0.82–1.55)
Harlow *et al.* (1992)	<10 1.2 (0.5–2.6)	<5 1.5 (0.8–2.7)
	10–29 1.6(1.0–2.7)	5–29 1.2 (0.6–2.2)
	≥ 30 1.6 (1.0–2.7)	≥ 30 1.8 (1.1–3.0)
Ness and Cottreau (1999)	1 2.0 (1.0–4.0)	NG
	1–4 1.6 (1.1–2.3)	
	5–9 1.2 (0.8–1.9)	
	10+1.2 (1.0–1.5)	
Whittemore *et al.* (1998)	1–9 1.60 (1.00–2.57)	1–20 1.27 (0.82–1.96)
	10+1.11 (0.74–1.65)	>20 1.45 (0.94–2.22)
Wong *et al.* (1999)	1–9 0.9 (0.6–1.5)	NG
	10–19 1.11 (0.74–1.65)	
	≥ 20 0.9 (0.6–1.2)	

CI, confidence interval; NG, not given; OR, odds ratio.

dose–response relationship. As outlined above, treatment for ovarian cancer may induce specific symptoms that could prompt short-term talc use. For instance, some early stage patients may undergo radiation therapy, which causes skin irritation. Such side effects could result in some patients using talc products to address these side effects. Talc is often recommended to keep skin folds in the perineum dry and prevent skin breakdown secondary to radiation. In addition, symptoms of the disease process itself could cause some women to use talc to counter these symptoms. Paulsen *et al.* (2005) and Golf *et al.* (2004) document that a number of symptoms are quite common among ovarian cancer cases versus control participants. For instance, Golf *et al.* show that increased abdominal size is over seven times more common among cases versus controls, whereas abdominal bloating is 2.5 times more common. The combination of bloating, increased abdominal size and urinary symptoms were found in almost half of all patients with ovarian cancer, but in only 8% of controls. In addition, of interest are the findings by Green *et al.* (1997) that increased ovarian cancer risk was seen among patients with painful periods or excessive vaginal bleeding. Again, such symptoms

could prompt talc use and lead to a spurious association with talc. Although there are no firm data in the existing literature to definitively establish that these factors lead to increased short-term use of talc, the scenarios are plausible and could explain the inverse dose–response relationship seen in a number of epidemiological studies.

The majority of reports largely ignore the counterintuitive findings, although Cramer *et al.* (1999) attribute the dose–response inconsistencies, possibly to the 'crudeness' of the exposure measurement used. What is not acknowledged is that this same problem of imprecise exposure estimates could also explain a spurious positive association of talc and ovarian cancer, especially in light of the inconsistent outcomes across reports. In summary, the failure to show a coherent and consistent relationship between talc exposure and ovarian cancer risk argues against a causal association.

An additional limitation of the existing literature dealing with the proposed talc/ovarian cancer association is the lack of any known biological mechanism through which talc particles could induce ovarian tumors. This represents probably the most troublesome aspect of arguments in support of this proposed causal association. It is also interesting to note that biological theories put forth to explain how talc may cause neoplastic transformation have changed over time as various proposed mechanisms have met with criticism in the developing literature.

Initially, Cramer *et al.* (1982) and others sought to draw an analogy between talc and fibrous asbestos, the latter being a known and well-described carcinogen. The biological effects of asbestos have been elucidated over the last 50–60 years by a multitude of epidemiological, in-vitro and in-vivo studies (Huncharek, 1986). Specific asbestos types are recognized as both animal and human carcinogens and, because of this fact, this commodity is banned from use in the United States.

A number of investigators initially implicated talc products as possible carcinogens, as before the early 1970s some talc products contained small amounts of asbestos fibers (Rohl *et al.*, 1976). Clearly, such products could possibly represent a carcinogenic risk secondary to the asbestos contamination. It should be pointed out that this in no way implicates talc as a toxin as the problematic constituent of such products was the asbestos fibers, not talc.

Since the early 1970s, the relevant industries voluntarily eliminated asbestos contamination from talc products. On account of this, the 'antitalc' argument shifted to implicate talc itself as a carcinogenic risk based on its 'chemical similarity' to asbestos. It is interesting, and confusing, as to why talc is thought by some to be carcinogenic based on the fact that there are some common chemical constituents of talc and asbestos.

Both commercial talc and the group of minerals known as asbestos are magnesium silicates. Beyond that fact, the two substances share no common characteristics. The work by Stanton *et al.* (1981) shows that the carcinogenic ability of fibrous asbestos is due to its structure, not its chemical composition. Although talc and asbestos are both magnesium silicates, they are structurally distinct and belong to different mineral groups and subgroups, as detailed by Muscat and Huncharek (2008). Amphibole asbestos minerals are inosilicates while talc is a member of the silicate subclass phyllosilicate and group clay or montmorillonite/smectite. Although serpentines, including serpentine asbestos (chrysotile), are also phyllosilicates, serpentine minerals belong to the kalolinite–serpentine group. The asbestos varieties of serpentine are structurally different from other members of the serpentines in that their brucite layers and silicate layers bend into tubes that produce fibers. Nonfibrous serpentine does not have carcinogenic properties and it is clear that the physical structure of serpentine asbestos (and amphibole asbestos) is responsible for its disease-causing potential, not its atomic constituents. It simply does not follow that one should assume talc is carcinogenic simply because it is a silicate. Structure, not chemical composition, dictates toxicity/carcinogenicity.

Given the dissimilarities between talc and asbestos with regard to their fibrous shapes, the weak but increased associations in the epidemiological studies could be attributed to other mechanisms, assuming that the statistical associations are unbiased and not due to confounding. Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate an inflammatory response (Ness and Cottreau, 1999). Pelvic inflammatory diseases, however, such as endometriosis, peritonitis, tuboovarian abscess formation, etc., have not been associated with an increased risk of ovarian cancer. A meta-analysis of studies of antiinflammatory drug use found no reduction in ovarian cancer risk (Bonovas *et al.*, 2005). In fact, the study by Merritt *et al.* (2008) that was cited by Epstein *et al.* also showed no relationship between inflammation and ovarian cancer risk.

Most recently, Cramer *et al.* (2005) proposed that the talc/ovarian cancer association might be explained by the induction of anti-MUC1 antibodies. This idea has been debated on statistical grounds in which talcum powder applied to the perineum was associated with increased anti-MUC1 expression but the correlation was also observed when talc powder was applied to other body parts. More importantly, the simple observation that talc elevates immunoglobulin protein levels in blood, possibly by heat shock proteins, seems to have no known direct relevance for ovarian cancer, as anti-MUC1 is associated with other cancers and because there is no known role of heat shock proteins in ovarian cancer risk.

Some of the most important biological data supporting the nontoxic nature of talc come from the clinical use of talc in treating both malignant and benign pleural effusions in humans (i.e. pleurodesis). This is a common procedure in the United States and elsewhere and talc slurry is applied directly to the pleura (through chest tube placement) to induce obliteration of the pleural space by scarring and prevent the reaccumulation of fluid secondary to tumor or benign causes. Multiple long-term clinical studies, as reviewed by Muscat and Huncharek (2008), have not shown a single case of cancer secondary to direct talc application to the human pleura (Shaw and Agarwal, 2004). There are also data showing that talc has demonstrated antitumor properties secondary to the induction of endostatin when used in pleurodesis (Najmunnisa *et al.*, 2007). In fact, patients with pleurodesis treated with talc are known to experience longer survival times than those treated with other sclerosing agents. This is likely due to the tumor-inhibitory effects of talc.

Finally, other human data, such as the demonstration that talc inhaled in mining and milling operations is not associated with increased pulmonary tumors, and the likelihood that talc could selectively induce ovarian cancer and not lung cancer at exposure concentrations orders of magnitude lower than that experienced in occupational settings, argue against its toxicity (Muscat and Huncharek, 2008).

Although the process of drawing causal inferences from scientific data is complex, application of accepted standards, as noted above, to the talc/ovarian cancer relationship clearly indicates that the available epidemiological and other evidence does not support a causal connection. The weak association shown in a subset of observational studies can potentially be explained by numerous alternative hypotheses, as detailed throughout this document. Given the lack of supporting evidence from in-vivo, in-vitro, and clinical research studies using human participants, the weak epidemiological association is unlikely to be causal.

Summary

Although separating causal from noncausal explanations for an observed statistical association is a difficult process, there currently exist commonly accepted guidelines by which such inferences can be made. These scientific approaches include consideration of the strength of the association, the consistency of the finding across studies, and existence of a biological explanation of the observed phenomenon, among others. When applied to the context of a proposed talc/ovarian cancer association, we conclude that the weak statistical associations cited in the petition do not support a causal association.

These conclusions are based on a number of statistical, methodological, and biological issues. First, contrary to the assertions of Epstein (2008), findings from the cited studies are not consistent from study to study, and also differ by study design. Two meta-analyses by Huncharek *et al.* (2003) and Langseth *et al.* (2008) both show significant differences in summary ORs between population-based and hospital-based case–control studies, with the latter showing generally null results. The Nurses Health Study, the one prospective study that examined this association, found no risk with talc dusting. Formal statistical tests for heterogeneity in both analyses support this finding. This fact suggests the existence of bias, and standard approaches to meta-analysis indicate that the pooled OR, in this case an OR of 1.30, is not valid in the presence of heterogeneity. Huncharek and Muscat (2007) suggest multiple possible sources of bias that could produce a spurious positive finding, including unaccounted for effects of cancer treatment and confounding by smoking.

The assembled data also fail to show a clear dose–response relationship, that is, increasing ovarian cancer risk with increasing talc exposure. Some epidemiological studies actually suggest an inverse association between perineal talc exposure and cancer risk. The reasons for this inverse association in some studies are not known, but could be due to aspects of talc usage that are not fully understood such as the possibility that disease symptoms or cancer treatment may spur temporary talc use in case patients.

There is no coherent biological explanation as to how talc could induce cancer of the ovary. The theories put forth to explain the statistical association between talc and ovarian cancer have changed over time with little underlying consistency. The long-standing claim that talc is chemically 'similar' to asbestos and is therefore a carcinogen is a misunderstanding of the chemical and physical properties of talc.

The use of therapeutic talc for pleurodesis in patients with benign and malignant pleural effusions involves the direct application of talc to the human pleura. Clinical follow-up studies of these patients have shown no increased incidence of lung or pleural malignancies despite patient follow-up extending over decades. The above-noted data are supported by the lack of positive findings among occupational cohorts exposed to talc, and negative findings from various animal studies. More recently proposed mechanisms based on other biological pathways are speculative at this point. Given the lack of supporting evidence from in-vivo and clinical research studies using human participants, the weak and inconsistent epidemiological associations, that also lack a gradient in effect, argue against a claim of causality.

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Conflicts of interest

Drs. Huncharek and Muscat were consultant to Johnson and Johnson Consumer Product Worldwide at the time initial drafts of this manuscript were produced.

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